Polychlorinated Biphenyls (Aroclor 1242): Effects of Uptake on *E. coli* Growth

by Julian E. Keil, M.S.*, Charles D. Graber, Ph.D.†, Lamar E. Priester, Ph.D.‡, Samuel H. Sandifer, M.D.*

Introduction

Polychlorinated biphenyls (PCBs), similar to DDT in structure and physiological effects (7), are of concern to the scientific community because of their ubiquity (8), persistence (11) and possible toxicity. The alleged lack of biodegradability of this environmental pollutant has caused alarm and curtailment of this useful chemical. Work as early as 1963 by Cope (3) indicated that DDT, another chlorinated hydrocarbon, might be metabolized by several species of bacteria. Subsequently, other workers (2, 4, 9, 10) reported degradation of this insecticide by bacteria, fungi, and marine diatoms. Unpublished work performed in our laboratory (5) also indicated possible metabolism of certain PCB components by *Cylindrotheca closterium*. With the thesis established that DDT was indeed biodegradable and with evidence that PCBs may be biologically converted, the authors postulated that human flora may play an important part in the metabolism of DDT, PCBs, and other ecological contaminants.

Experiments were performed to study the effects of PCBs in vitro on a facultative organism, *E. coli*, common to human intestinal flora. This bacterium was also selected because it is the prime indicator of fecal contamination.

Materials and Methods

The study organism, *Escherichia coli*, was selected, because it is a common autochthonous, human intestinal aerobe, easily cultured, and is an accepted standard indicator of fecal contamination

Initial work screened a wide range (0–1000 ppm) of PCB concentrations, against *E. coli* by use of impregnated paper filter discs similar to those used to test antibiotic sensitivity. Impregnated discs with PCB concentrations of 0, .001, .01, .1, 10, 100, and 1000 ppm were placed on tripticase soy broth (TSB) agar plates which had been inoculated with *E. coli*. Experimentation indicated that 6 mm diameter discs cut from number 2 Whatman filter paper would absorb .02 ml of the acetone solvent. The discs were dried prior to use.

For the first experimental series (I), 20 one-liter Erlenmeyer flasks, each with 500 ml. TSB, were inoculated with *E. coli*. Four flasks served as controls, 4 for each of 2 levels of PCB treatments, and 4 as acetone controls since acetone was the suspending vehicle for the toxicants. Additionally, 4 flasks were used as uridine controls. One flask, containing only media, was used as a growth check. Each flask was vigorously agitated every 2 hours during the first and last 8 hours of culture time.

At the conclusion of a 24-hour incubation period, flask contents were centrifuged, the resulting pellet lyophilized, weighed and assayed for PCBs, nucleic acids and tritiated uridine (^3H_U) uptake.

A second experiment (Series II), identical in treatments except for uridine, was conducted to confirm stimulation patterns observed in Series I. Series II measured lyophilized harvest weights only.

A third trial (Series III), identical in design to

* Preventive Medicine Section, Medical University of South Carolina, 80 Barre Street, Charleston, S. C. 29401.

† Microbiology Department, Medical University of S. C.

‡ Environmental Health Laboratories, State Board of Health, Columbia, S. C.

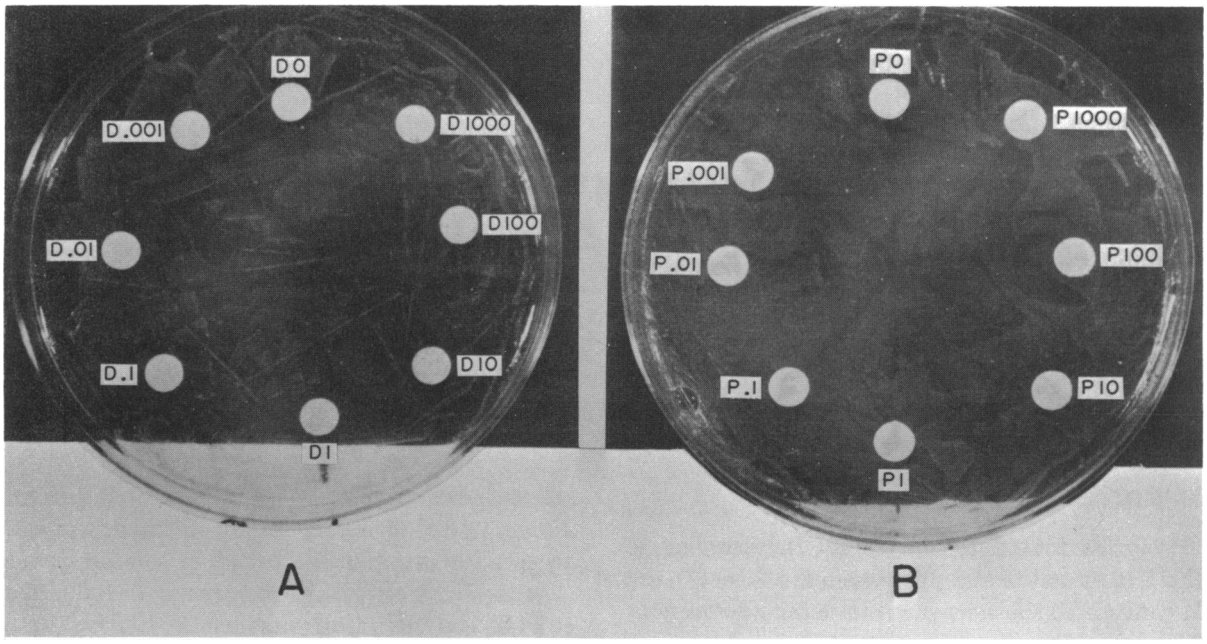

FIGURE 1. Sensitivity screening of *E. coli* on TSB to 0, .001, .01, .1, 1, 10, 100, and 1000 ppm of A, DDT; B, PCB.

Series I, assayed tritiated uridine uptake after 5 hours and 8 hours incubation time to determine if assimulation rates were affected by PCBs during the logarithmic growth phase. Aroclor 1242 (PCB—42% chlorine) was used as the source of active ingredients in these experiments.

Samples of bacteria were extracted with nanograde hexane and a 5 μl aliquot was injected into the gas chromatograph for analysis.

Two-column systems were used: a 6 foot × ¼ inch O.D. glass column packed with 3% SE-30 on 60–80 ABS chromosorb W and 2% QF-1 on 60–80 ABS chromosorb W. The operating parameters were: (1) inlet temperature 235°C; (2) column temperature 200°C isothermal; (3) detector temperature 240°C; (4) flow-rate 70 cc per/min. N_2.

Table 1. Lyophilized Weights and Toxicant Uptake by *Escherichia coli* Cultures Exposed to PCB (Bacterial Series I).

Treatment	Mean[1] harvest weights (mgm)	Mean[1] residue (μg/g)
PCB .1 μg/ml	86.0	13.6
PCB .01 μg/ml	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD_{05}[2]	13.6	10.8

[1] Mean of four replicates.

[2] LSD_{05} = Least significant at 95% probability level calculated from one way analysis of variance.

Qualification was accomplished by measurement of the absolute and relative retention times of 5 major peaks of the PCBs. Quantitation was accomplished by determination of the area under the curve of the five major peaks. Utilizing this technique, recoveries of 92.4% of the PCBs were obtained.

RNA and DNA were measured using a modification of Agranoff's procedure (1). Radio assays utilized the Nuclear-Chicago Mark I Liquid Scintillation Spectrometer.

Results and Discussion

As may be seen from Fig. 1, concentrations up to 1000 ppm of PCBs impregnated into filter paper discs failed to inhibit *E. coli* growth on TSB agar.

As shown in Table 1 (Series I), the addition of .01 and .1 μg/ml PCBs to TSB markedly stimulated *E. coli* growth above control levels. This is consistent with other microorganism work (4, 6) showing that DDT and PCBs are concentrated up to 1000 times by marine diatoms. The data also suggest that the concentration factor may be inversely related to dosage.

Table 2 presents data from a duplicate experiment (Series II), wherein only harvest weights were studied. This second series confirmed apparent stimulatory effects of all levels of PCBs.

There were no differences between treatments in DNA and RNA levels after 24 hours' incubation. The significance of this is not clear, since it had been anticipated that increased RNA levels would accompany growth increases. However, it is thought that differences may have occurred earlier than the 24-hour time.

Data showing uridine uptake by PCB-treated *E. coli* at several phases of growth are presented in Table 3. No significant differences in uptake between treatments and controls were evident except at 5 hours incubation time, when there was a significant diminution of ^3H_U uptake in PCB-dosed bacteria. While not consistent with increased culture yields, the lack of change in uptake between chemically treated *E. coli* and controls does coincide with chemical assays indicating no increased levels of nucleic acids.

Results from these experiments indicate that PCBs stimulate in vitro growth of *E. coli*. If this phenomena occurs in vivo further concern is justified since any environmental contaminant which affects indicator organisms may result in a distorted portrayal of the actual situation.

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Table 2. Lyophilized Weights of *Escherichia coli* Cultures Exposed to PCB (Bacterial Series II).

Treatment	Mean[1] harvest weights (mgm)
PCB .1 μg/ml	186.4
PCB .01 μg/ml	153.2
Control	160.8
Acetone control	133.6
LSD_{05}[2]	23.6

[1] Mean of four replicates.

[2] LSD_{05} = Least significant difference at 95% probability level calculated from one way analysis of variance.

Table 3. Tritiated Uridine Uptake by PCB Treated *Escherichia coli* Cultures During Exponential and Decline Growth Phases.

Treatment	Counts per minute[1]		
	5 Hours	8 Hours	24 Hours
PCB .1 μg/ml	4342	2250	2192
PCB .01 μg/ml	4756	2367	2462
Uridine control	8020	2675	2389
LSD_{05}[2]	2800	979	859

[1] Mean of four replicates.

[2] Least significant difference at 95% probability level calculated from one way analysis of variance.

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